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Improving carnation resistance to root-knot nematode infection under greenhouse conditions

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ABSTRACT

The current research was conducted at Tissue Culture laboratory of Vegetables and Ornamental Dept., and greenhouse of Nematology Research Unit, Agricultural Zoology Dept., Faculty of Agriculture, Mansoura Univ., Egypt. The effect of certain components i.e. L-glutamic acid, ascorbic acid, yeast extract and phenylalanine introduced singly at 1 g/ L added to MS medium used for the growth of the carnation seeds, obtained from France before transplanting into plastic pots filled with sterilized loamy sandy soil on *Meloidogyne incognita* infection was evaluated under greenhouse conditions at 22±5°C. Results indicate that among tested treatments, application of L-glutamic acid gave the highest reduction percentage in *M. incognita* root galls by 57.14% while ascorbic acid ranked first in respect to egg-masses with value of 65.25%. However, Yeast extract exceeded other tested components in percentage increase of the whole plant fresh and shoot dry weights of the infected carnation seedlings and uninfected plants as well with values of 99.09 and 53.33%; and 55.96 and 211.8%, respectively. Thus, it could be concluded that yeast extract or L-glutamic acid applied in MS medium considered the best treatments in improving growth of carnation plants, increasing growth and suppressing *M. incognita* development and reproduction under greenhouse conditions.

INTRODUCTION

Carnation (*Dianthus caryophyllus*) is the most diffused cut-flower crop in protected cultivation in the Mediterranean areas, due to its suitability to environmental and climatical conditions. Carnation is a semi-hardy perennial treated as an annual plant. It is one of 300 species of annual and perennial herbs in the genus Dianthus and a member of the caryophyllaceae family. Carnation flowers are available year-round with a lot of colors and sizes, and frequently has new cultivars.

In Egypt, root-knot nematodes, *Meloidogyne* spp. are becoming a real threat to almost all ornamentals including carnation, especially in the newly reclaimed areas and they have been considered limiting factors in crop and flower production. Ibrahim *et al.* (2000) and Mostafa (2012). Johnson *et al.* (2003) mentioned that carnation is susceptible to root-knot nematode infection. Because of the lack of resistance in most plants to most species of root-knot nematode as well as the environmental restrictions on nematicidal use for controlling plant parasitic nematodes; biological control and other ecofriendly disease control methods have gained recently increasing interest.

The use of induced resistance in plants could offer a considerable potential for biological control Deverall (1995). Thus, a new strategy for controlling plant parasitic nematodes is based on the activation of the plant's

own defense system via various biotic and abiotic agents. The pretreatment of plant with an inducing agent (organism or compound) appears to illicit the plant to amount an effective defense response upon subsequent encounters with pathogen converting what would have been a compatible interaction to an incompatible one.

The practice of plant tissue culture has contributed towards the propagation of large number of plants from small pieces of stock plants in relatively short period of time (Daniel 1998). Rapid and easy mass production also allows for improvement of selections of plant with enhanced stress or pest resistance. However, during the early transplanting stages, carnation tissue culture plantlets need higher level of care and attention than conventional plants. In current study, producing healthful seedlings that tolerate or resist the infection of plant parasitic nematodes is a focal target.

In recent years, there has been an increased interest in the application of antioxidants, vitamins, growth hormones and natural products to medicinal and agricultural treatments Wolucka *et al.* (2005); Noweer *et al*. (2005); Saeed (2005) and Nour El-Deen *et al.* (2013). In 2008, Nour El- Deen succeeded to produce healthful tomato seedlings that tolerate the root-knot and lesion nematodes infection by using certain components in MS media. Trials to control nematodes affecting ornamental plants produced by tissue culture by altering the chemical components of growth medium to produce seedlings able to sustain nematode infection are lacking**.**

Therefore, the aim of this study was to investigate whether the application of growth medium supplemented with certain components would induce the resistance or tolerance of carnation plant to root-knot nematode.

MATERIALS AND METHODS

The current research was conducted at tissue culture laboratory of Vegetables and Ornamental Plants Dept., and greenhouse of Nematology Research Unit(NERU), Agricultural Zoology Dept., Faculty of Agriculture, Mansoura Univ., Egypt.

Seeds germination:

The carnation double mixture seeds used in this study were obtained from France. Seeds of plant were washed with tap water containing a few amount of household detergent for 10 min.,then seeds were the surface sterilized by immersing in ethanol 70% plus a drop of Tween 20 for 30 sec. followed by 30% sodium hypochlorite (NaOCl) solution plus a drop of Tween 20 for 5 to 7 min. Seeds were rinsed three times in sterilized distilled water in laminar air flow hood to remove the residuals, and cultivated on autoclaved watered-cotton as a liquid medium in 250 ml jars under aseptic conditions. Ten seeds were distributed randomly in each jar, kept in growth chamber at 25± 2°C with 16 h. light/8h. dark cycle and left for germination for 1-2 weeks.

Preparation of the media:

Full strength MS (Murashige and Skoog, 1962) media (4.4 g/l) was prepared and supplemented with certain components i.e. ascorbic acid as vitamin or antioxidant, yeast extract as natural product and phenylalanine and

L-glutamic acid as amino acids at 1 g/ L. The media were distributed into 1000 ml jars for each tested components, contained 600 ml of prepared media each. Twenty jars (250 ml) without any of the previous compounds were served as control. The media were poured into 250 ml jars, with 30 ml of the medium enriched with the tested materials each. The pH was adjusted to 5.7 before autoclaving. The media were then solidified with 8 g/l agar and sucrose at 30 g/l. Then, the media were autoclaved for 15 min. at 121º C, 1.5 kg/cm3 and left for three days to follow the contaminated jars. One week old sterilized carnation plantlets maintained on sterilized watered-cotton were transferred on MS medium supplemented with BAP (6-benzyl aminopurine) at 0.5mg/L then cultured in the previous media after two weeks. Each treatment of the tested components has twenty jars as replicates, contained one explant each, kept inside the growth chamber at 25°C under the system of 2000 Lux. fluorescent lamps for 16 h. light and 8 h. dark cycle for three weeks.

Plantlet acclimatization:

The in-vitro rooted plants were taken out from the medium after 4 weeks, washed in warm tap water to get quit of any medium residues to remove a potential source of contamination. Thereafter, the plantlets were transplanted into black plastic pots (one seedling/pot 5-cm-d.) filled with peat moss: Vermiculite (1:1) which were autoclaved at 121°C and pressure of 1.2 kg/cm2 for 20 min. for acclimatization. The objective of this stage was to obtain well-adapted carnation plants for the following evaluation of pots performance ex-vitro.

Potted plantlets were transferred to glass containers (100x60x25 cm) existed in a growth room. Tap water was added to the bottom of glass containers as a thin layer (0.5 cm in height). The glass containers were kept covered with a clear polyethylene sheet to maintain high relative humidity and were incubated at 25± 2°C under constant fluorescent light of 2500 Lux for 16 h. light/8h. dark photoperiod cycle in the incubation room for fourteen days. At the end of incubation period (14 days), the clear polyethylene sheet was completely raised on glass containers, thereafter, potted plants were transferred outside the growth room to the internal space area of laboratory for 7 days. During this period, the plants were watered every 2 days with tap water and once with compound fertilizer which consisted of N:P:K (19:19:19) + Micro elements at 1.0 g/l of irrigation water. After 3 weeks of ex-vitro growth, fifty plants were transferred to the greenhouse, and repotted in black plastic pots (16-cm-d.) containing sterilized sand and loam soil (1:1 v:v).

Nematode inoculum:

The root-knot nematode, *M. incognita* eggs were extracted from infected coleus (*Coleus blumei*) roots after washing free of soil, cut into small pieces by using 0.5 % NaOCl solution and shaking for 2 minutes (Hussey and Barker, 1973). These eggs were obtained from a pure culture established from a single egg-mass of *M. inc*ognita that previously identified according to the characteristics of its perineal pattern (Taylor and Sasser, 1978) and reared on coleus plants grown in greenhouse of Nematology Research Unit(NERU), Agricultural Zoology Dept., Faculty of Agriculture, Mansoura

University, Mansoura. Nematode inocula consisted of 2000 viable eggs of *M. incognita*.

Experimental Design:

Twenty five seedlings (70 days old) of carnation plants were separately inoculated with 2000 viable eggs of *M. incognita* . Of them five seedlings were left free from any treatment and served as control (Ck1). Another twenty five seedlings treated with the previous materials were left free of nematode inoculation. Of them five seedlings were left free from any treatment and served as control(CK2) Therefore the treatments for both infected and uninfected plants were as follows:

1) Ascorbic acid,
2) Yeast extract,
3) Phenylalanine,
4) L – glutamic acid,
5) Untreated and inoculated plants (Ck1) and
6) Untreated and uninoculated (N alone) (Ck)

Each treatment was replicated five times. Pots were then randomly arranged on a greenhouse bench at 22±5°C. Plants were watered regularly as needed. Forty five days after nematode inoculation, plants were harvested. Data dealing with:

1. Lengths of shoots and roots.
2. Fresh weights of shoot and root.
3. Dry weight of shoot.
4. Numbers of branches and leaves

Infected carnation roots were stained in 0.01 lactic acid fuchsin and examined for the numbers of galls and egg-masses (Byrd *et al*., 1983). Root gall index (RGI) and egg-mass index (EI) were determined according to the scale given by Taylor and Sasser (1978) as follows: 0= no galls or egg-masses, 1= 1-2 galls or egg-masses, 2= 3-10 galls or egg-masses, 3= 11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5= more than 100 galls or egg-masses. Statistically, data were subjected to analysis of variance (ANOVA) by the general linear models (GLMs) procedure using (SAS) SAS Institute (1994). Mean comparisons were performed using the least significant difference (LSD) method according to Gomez and Gomez (1984). A significant level of 0.05 was adapted for all statistical analyses.

RESULTS AND DISCUSSION

Data presented in Tables (1, 2 and 3) illustrate the impact of certain components i.e. ascorbic acid, yeast extract, phenylalanine and L-glutamic acid added to MS medium on controlling *M. incognita* infection, its development and the resulting effect on carnation plant growth parameters either infected or uninfected under greenhouse conditions. Data in table (1) verify that all tested materials applied in MS medium to carnation seedlings infected with *M. incognita* were significantly effective in reducing numbers of formed galls and egg-masses when compared with those of the inoculated untreated check. It is interesting to notice that L-glutamic acid as amino acid

was the ultimate efficacious treatment performing crucial reduction in number of galls by 57.14% followed by ascorbic acid (44.13%), whereas, yeast extract was the least one in this respect with reduction percentage of 16.51%. On the other hand, the highest reduction percentage of egg-masses resulted by ascorbic acid treatment with value of 65.26%, followed by L-glutamic acid with value of 53.05%. Similar trend was obviously evident in the case of indices of root galls and egg-masses number as well. The lowest values were accomplished by L-glutamic acid (3.4 and 3.0) and ascorbic acid (3.8 and 2.8) respectively, whereas, the highest values of root gall index was recorded with yeast extract which was on par (4) with nematode alone, (Table 1). These results agreed with Saeed (2005) in respect to amino acids and vitamins on root-knot nematodes who reported that high and significant percentage of reduction in nematode counts on soybean was obtained by vitamin-C and E at 250 and 500 ppm conc.

Table (1): Development and reproduction of *Meloidogyne incognita* infecting carnation seedlings as influenced by the addition of certain components to MS medium then transplanted to sterilized soil under greenhouse conditions.

Each value is the mean of five replicates . N= 2000 eggs of *M. incognita

Treatments	*Galling and Reproduction response					
	Root galls			Egg-masses		
	No.	Reduction %	**RGI	No.	Reduction %	**EI
Ascorbic acid	35.20bc	44.13	3.80ab	14.80c	65.26	2.80c
Yeast extract	52.60ab	16.51	4.00a	23.00bc	46.01	3.00c
Phenylalanine	45.20bc	28.25	3.80ab	28.20b	33.80	3.60b
L-glutamic acid	27.00d	57.14	3.40b	20.00bc	53.05	3.00c
Check medium (N alone)	63.00a	0.0	4.00a	42.60a	0.0	4.00a
L.S.D 0.05	11.78		0.52	8.28		0.39

****Root gall index (RGI) and egg-mass index (EI) according to the scale given by Taylor and Sasser (1978) as follows: 0= no galls or egg-masses, 1= 1-2 galls or egg-masses, 2= 3-10 galls or egg-masses, 3= 11-30 galls or egg-masses, 4= 31-100 galls or egg-masses and 5= more than 100 galls or egg-masses.**

Means followed by the same letter do not differ significantly (P< 0.05) according to Duncan's multiple range test.

Reviewing data presented in Tables (2 and 3) reveal that medium supplemented with tested components i.e. ascorbic acid, yeast extract, phenylalanine and L-glutamic acid in comparison with check medium had more or less effects on growth of carnation plants infected with *M. incognita* and the uninfected ones as well. Data in Table (2) demonstrated that all of tested materials were found to be effective in protecting and improving carnation plant growth infected with nematode to certain extent. It is clear that all of the tested components significantly improved length of the infected carnation plant as compared with those of the check ones. Carnation plants infected with *M*. *incognita* and treated with ascorbic acid resulted in a significant improvement in plant length (37cm) compared to untreated inoculated plant which gave the shortest length (23.4 cm).

Treating plants with yeast extract significantly increased drastically fresh weight of the whole plant by 99.09% when compared with other treatments and control followed by L-glutamic acid (39.35%) then phenylalanine (17.87%). Comparatively, treatment of yeast extract overwhelmed other tested treatments in the increment percentage of shoot dry weight since its value averaged 53.33% followed by L-glutamic acid (18.67%), whereas ascorbic acid treatment gave the least value in this parameter (12.44%). Plants treated with phenylalanine and ascorbic acid gave the highest number of branches with percentage increase of 104.35 and 91.30% as well as number of leaves (150.4 and 147.8), respectively without significant difference between them whereas, carnation plants maintained on check medium had the lowest values of the same parameters (Table 2).

Data in Table (3) showed that carnation plants free of nematode infection and treated with ascorbic acid resulted in the greatest significant increase in total shoot and root length than the three other examined components i.e. phenylalanine, yeast extract and L-glutamic acid with values of 41.6, 35.0, 34.2 and 32.2 cm, respectively. Although, most tested materials improved fresh weights of the uninfected plants, some of which had phytotoxic effects. For instance, treating with L-glutamic acid, decreased value of total plant fresh weight in relative to those of plant free of nematode and any treatment (check medium), since its value were amounted to -0.03%. Using yeast extract significantly increased fresh weight of the whole plant by 55.96% followed by phenylalanine (22.49%) then ascorbic acid (5.7%). The same trend was noticed regarding shoot dry weight, since plants treated with yeast extract significantly increased shoot dry weight values by 211.8% followed by phenylalanine (110.24%) whereas, L-glutamic acid treatment ranked the least value for this concern which averaged to 77.17%. It was surprise to note that most of the tested components reduced the number of branches on carnation plant. However, ascorbic acid as vitamin gave the highest numbers of branches (25.60) and leaves (235.80) as reported in Table (3).

The obtained results prove that vitamins may share partially in inducing resistance in susceptible plants. Vitamin C (ascorbic acid), in particular could success fully be used in plants to elevate their resistant ability. Mode of action of such vitamin and its role in inducing resistance in plants against nematode infection has been explained by Arrigoni *et al.* (1976& 1979). These findings are in accordance with those previous reported by Nour El-Deen *et al.* (2008) who observed that the addition of castor and garlic oils, ascorbic acid and L-glutamic acid traces to MS medium was more effective to protect and produce healthful tomato seedlings against the infection of *M. incognita*.

2-3

As for all the components tested, it is clear that fresh weighs of whole carnation plants were higher in infected than uninfected ones. The nematicidal activity of the tested biotic factor i.e. yeast extract and abiotic agents i.e ascorbic and L-glutamic acids as fertilizers as well as their thermostable toxin in the management of endoparasitic nematode, *M. incognita* on carnation plants can be varied from component to another. These variations may be attributed to differences in chemical, nature compound present in these tested material and method of application used. The safety of such materials and its low cost is one of its advantage. Although yeast extract treatment gave the highest values of growth parameters in carnation, L-glutamic acid and ascorbic acid were the best in reducing nematode infection. Usage of such components has gained great benefits in eliminating nematode development and minimizing host damage. These materials have exhibited more interest as resistance inducers in plants against the pathogenic nematodes i.e. *M. incognita*. The obtained results have focused the attention towards their role on nematode management, since they may be involved in plant metabolism for synthesizing of bioactive compounds that can oppose nematode development and reproduction.

Obviously, results of this investigation indicated that the possible use of these components at low cost to MS medium of plant tissue culture will avoid the pollution of the Egyptian agriculture environment from using chemical nematicides when added directly to soil or spray on plants against *M. incognita* infecting such economic plant.

REFERENCES

Arrigoni, O., G. Zacheo; R. Arigoni-Liso; T. Belever Zacheo and F. Lamberti (1979). Relationship between ascorbic acid and resistance in tomato plants to *Meloidogyne incognita*. Phytopathology, 69: 570-581.

Arrigoni, O.; R. Liso-Arrigoni and G. Colabrese (1976). Ascorbic acid as a factor controlling the development of cyanide-insensitive respiration. Science, 194: 332-333.

Byrd, D. W.; T. Kirkpatrick and K. Barker (1983). An improved technique for clearing and staining plant tissues for detection of nematodes. J. Nematol.,15(3):142-143.

Daniel, R. L. (1998). The many dimension of plant tissue culture research. Webmaster of Aggie Horticulture publications, pp:201-210.

Deverall, B. J. (1995). Plant protection using natural defense systems of plants. In: (Eds. Andrews, J. H. & Tommerup, I. C.). Advances in plant pathology, vol. 11. San Diego, USA, Academic Press, pp. 211-228.

Gomez and Gomez (1984). Statistical procedures. Agric. Res. 2nd Ed. Johnwiley and Sons, Inc, New York, USA.

Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reptr. 57:1025–1028.

Ibrahim I. K. A.; Handoo; Z. A. and El-Sherbiny, A. A. (2000). A survey of phytoparasitic nematodes on cultivated and non-cultivated plants in Northwestern Egypt. Suppl. J. Nematol., 32(4):478-485.

Johnson, S. B. N.; Cannayane, I. and Rajendran, G. (2003). Studies on the pathogenic level of *Meloidogyne incognita* on Gladiolus and Carnation. Current Nematol. 14(1/2):75-78.

Mostafa, M. H. (2012). Studies on protecting certain economic plants against root-knot nematodes *Meloidogyne spp.* infection by some medicinal and aromatic plant products. M.Sc. Thesis. Fac. Agric., Mansoura Univ.128pp.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15 (3): 473-497.

Nour El-Deen, A. H. (2008) Potential use of tissue culture technology in controlling plant parasitic nematodes. Ph. D. Thesis, Fac. Agric., Mansoura Univ.152 pp.

Nour El-Deen, A.H.; Omaima M. Abdel-Kafie and Naira M. El-Ghareb (2013). Evaluation of seaweed extract and various plant products against *Meloidogyne incognita* on basil. Georgikon for Agriculture, 16 (1): 29-34.

Noweer E. M. A. and Susan A. A. Hasabo (2005). Effect of different management practices for controlling root-knot nematode *Meloidogyne incognita* on squash. Egypt. J. Phytopathol, 33(2): 73-81.

Saeed, M. R. M. (2005). Utilization of some specific materials to stimulate resistance in some host plants against the root-knot nematodes. Ph. D. Thesis, Cairo Univ., Fac. of Agriculture, 220 pp.

SAS Institute (1994). SAS/STAT User's Guide: Statistics. Vers. 6.04, 4th Ed., SAS Institute Inc., Cary, N. C., USA.

Taylor, A.L.; and J. N. Sasser (1978). Biology , identification and control of root-knot nematodes (Meloidogyne species).Coop.Publ,Dept.Plant Pathol.,North Carolina State Univ., and U.S.Agency Int.Dev.,Raleigh,NC.,111pp.

Wolucka, B. A.; A. Goossens and D. Inze (2005). Methyl jasmonate stimulates the de novo biosynthesis of vitamin C in plant cell suspensions. Journal of Experimental Botany, 56 (419):2527-2538.

تحسين مقاومة القرنفل للإصابة بنيماتودا تعقد الجذور تحت ظروف البيوت المحمية

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****جامعة المنصورة – كلية الزراعة- قسم الحيوان الزراعي**

تم اجراء هذا البحث في معمل زراعة الأنسجة بقسم الخضر و الزينة ، والصوبه السلكية بوحدة بحوث النيماتودا ، قسم الحيوان الزراعي ، كلية الزراعة ، جامعة المنصورة ،مصر لدراسة تأثيراضافة بعض المكونات مثل حمض الجلوتاميك ،حمض الاسكوربيك ، مستخلص الخميرة و الفينيل الالنين منفرده بمعدل ١جم/لتر لبيئة موراشيج و سكوج و المستخدمة لتنمية بذور القرنفل الخليط المزدوج و التي تم الحصول عليها من فرنسا .تم زراعة الشتلات الناتجة في اصص بلاستيكية تحتوي علي خليط من التربة المعقمه (١ طمي : ١ رمل ، حجم:حجم) مصابة وغير مصابة بالنيماتودا . اجريت العدوى بنيماتودا تعقد الجذور بمعدل ٢٠٠٠بيضة لكل نبات وتم حصاد التجربة بعد مرور ٤٥ يوم من العدوى تحت ظروف الصوبه ٣٠ ±٥ درجه مئويه.

و تشير النتائج بين المعاملات المختلفة الى ما يلى:

١- اعطى الحمض الامينى ل-جلوتاميك اعلي نسبة انخفاض في اعداد العقد الجذرية ٥٧,١٤%

٢- جاء حمض الاسكوربيك في المركز الاول بالنسبه لكتل البيض بنسبة ٦٥,٢٥% .

٣- تفوق مستخلص الخميره علي المعاملات الاخري في النسبه المئويه للزياده في الوزن الطازج و الجاف لنباتات القرنفل المصابه و الغير مصابه بقيم ٩٩,٠٩ و ٥٣,٣٣% و٥٥,٩٦ و ٢١١,٨ % علي التوالي .

وبالتالي يمكن ان نوصي بأضافة مستخلص الخميرة او ل-حمض الجلوتاميك الي بيئة موراشيج و سكوج كافضل معاملات لتحسين نمو نبات القرنفل و زيادته و تقليل تطور نيماتودا تعقد الجذور و تكاثرها في التربة المصابة.

قام بتحكيم البحث

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Table (2): Effect of certain components added to MS medium on the growth response of carnation seedlings transplanted to sterilized soil and infected with *Meloidogyne incognita* under greenhouse conditions.

Treatment	*Plant growth response											
	Length (cm)		Fresh weight (g)		Fresh wt. of the whole plant (g)	Inc. %	Shoot dry weight (g)	Inc. %	Number of anches	Inc. %	Number of leaves	Inc. %
	Shoot	Root	Shoot	Root								
Ascorbic acid	26.40a	10.60ab	12.67ab	26.02c	38.69c	13.36	2.53b	12.44	17.60a	91.30	147.80a	27.63
Yeast extract	18.00bc	8.00c	14.52a	53.43a	67.95a	99.09	3.45a	53.33	11.40b	23.91	120.20a	3.80
Phenylalanine	21.80b	9.20bc	13.03ab	27.20c	40.23bc	17.87	2.66b	18.22	18.80a	104.35	150.40a	29.88
L-glutamic acid	19.60bc	11.80a	12.05b	35.51b	47.56b	39.35	2.67b	18.67	12.00b	30.43	129.80a	12.09
Check medium	15.80c	7.60c	11.01b	23.12c	34.13c	0.0	2.25b	0.0	9.20b	0.0	115.80a	0.0
LSD 5%	4.37	1.83	2.04	7.05	7.85		0.65		4.84		48.44	

*Each value is the mean of five replicates.
Means in each column followed by the same letter (s) did not differ at P< 0.05 according to Duncan multiple- range test.

Table (3): Effect of certain components added to MS medium on the growth of carnation seedlings transplanted to sterilized soil and uninfected with *Meloidogyne incognita* under greenhouse conditions.

Treatment	*Plant growth response											
	Length (cm)		Fresh weight (g)		Fresh wt. of the whole plant (g)	Inc. %	Shoot dry weight (g)	Inc. %	Number of branches	Inc. %	Number of leaves	Inc. %
	Shoot	Root	Shoot	Root								
Ascorbic acid	26.20a	15.40a	12.12b	23.69bc	35.81bc	5.70	2.41b	89.76	25.60a	11.30	235.80a	100.85
Yeast extract	23.20ab	11.00b	17.63a	35.21a	52.84a	55.96	3.96a	211.8	12.20bc	-46.96	104.20c	-11.24
Phenyl alanine	22.40abc	12.60ab	10.94b	30.56ab	41.50b	22.49	2.67b	110.24	15.60b	-32.17	173.60b	47.87
L-glutamic acid	20.20bc	12.00b	11.54b	21.28c	32.82c	-0.03	2.25b	77.16	10.60c	-53.91	128.60bc	9.54
Check medium	17.80c	10.40b	10.03b	23.85bc	33.88bc	0.0	1.27c	0.0	23.00a	0.0	117.40c	0.0
LSD 5%	4.85	3.06	2.82	7.05	8.64		0.86		4.45		45.17	

*Each value is the mean of five replicates.
Means in each column followed by the same letter (s) did not differ at P< 0.05 according to Duncan multiple- range test.